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Chromatographic and spectroscopic analysis of traditional medicinal plant *Melia azedarach* L. stem bark extracts

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Abstract

Melia azedarach L. is a medicinal plant belonging to the family meliaceae. It is an important traditional medicinal plant employed in indigenous system of medicine against several diseases. The current work determines the various bioactive compounds present in stem bark extracts (petroleum ether, acetone, ethanol and methanol) of *M. azedarach*. Chromatographic (TLC, CC, GC-MS) and spectroscopic (UV-visible and FT-IR) analysis were carried out to determine the bioactive compounds. The findings of thin layer chromatographic study revealed visible spots in varying solvent system with different R_f values. Based on the polarity, R_t and R_f value, methanolic extract was found to be best with three visible spots (0.05, 0.10 and 0.25) among the extracts tested. The column chromatographic analysis showed a maximum of 7 fractions in hexane: methanol (8:2) solvent run ratios with different colours. Two major compounds 9, 12-tetradecadien-1-o1 and 2-Cyclohexyl-4a,7-dimethyl-3,4,4a,5,6,8a-hexahydro-2H-benzoxaine-3-carboitrile have been detected through GC-MS analysis based on retention time. The UV-VIS spectrum profile confirmed the presence of halo compounds, alkyl aryl ether, amine, sulfate, alkanes, alcohols and aliphatic primary amine. The present work authenticates that, the stem bark extract of *M. azedarach* is found to hold important phytocompounds and serve an ultimate role in further experimentation on its pharmaceutical importance.

Keywords: *Melia azedarach*; bioactive; compound(s); chromatographic; spectroscopic; analysis; extract(s); stem bark

Introduction

Medicinal plants are widely renowned for its therapeutic importance and aids as a source of bioactive compounds in the pharmaceutical field [1]. Plants synthesize variety of chemical compounds possessing enormous medicinal properties (active principles), which is widely used in traditional and modern system of medicine in treatment of various diseases. The biological property of medicinal plants depends on the presence of primary and secondary metabolites, their biosynthetic origin and its functional groups. Primary metabolites include chlorophyll, amino acids, nucleotides, carbohydrates produced due to metabolic processes like photosynthesis, respiration and nutrient assimilation, whereas, secondary metabolites are alkaloids, tannins, flavonoids, phlobatannins, saponin and cardiac glycosides are products of secondary metabolism in plants [2]. These phytocompounds are reported in many plants which include *Pongamia pinnata* [3], *Mangifera indica* [4], *Moringa oleifera* [5], *Cissus populnea* [6], *Cymbopogan citratus* [7] to possess various biological activities with pharmaceutical importance

Melia azedarach L. is an important ethnomedicinal tree with wide range of biological activities and interesting bioactive constituents. It belongs to the family meliaceae, locally known as 'malai vembu' and commonly known as Indian lilac [8]. The plant is a fast-growing deciduous tree that can reach height of around 45 meters, native to India and China, it also found in Africa, America, Australia and Arab countries [9]. Traditionally, different parts such as leaf, flower, seed, fruit, bark and young branches have been used in the treatment of various ailments [10]. Preliminary phytochemical screening of *M. azedarach* showed the presence of number of phytoconstituents i.e., terpenoids, flavonoids, steroids, acids, anthraquinones, alkaloids, saponins, tannins [11, 12]. In addition, the plant possesses various pharmacological properties such as antioxidant, anticancer, antiviral, antimalarial, antibacterial, antifungal, and antifertility activities [13]. They are also used to treat skin diseases like scabies, brushing teeth, loosening or pain of tooth, rheumatic pain and fever; it also has insecticidal properties [14]. Furthermore, *M. azedarach* is used to treat burns, acts as mouth wash for gingivitis; pyrexia and bloody piles hysteria [15, 16] snake bite [17], diabetes, blood purifier and used to cure pimples [18].

The present scientific investigation was undertaken to analyze the phytocompounds by extraction, fractionation and isolation of chemical constituents from stem bark of *M. azedarach* through chromatographic and spectroscopic techniques.

Materials and Methods

Taxonomic Classification of *Melia azedarach* L.

Kingdom - Plantae
Division - Magnoliophyta
Class - Magnoliopsida
Subclass - Rosidae
Order - Sapindales
Family - Meliaceae

Genus - *Melia*
Species -*azedarach*
Botanical name - *Melia azedarach* L.

Collection and preparation of bark extracts
Stem bark of *M. azedarach* was collected from different regions of Tiruchirappalli district. The fresh bark was dried under shade room temperature for several days, powdered using electric grinder. 15 g of fine bark powder was mixed with 100 ml of each solvent such as petroleum ether, acetone, ethanol and methanol in a closed container and kept in magnetic stirrer for 3 days at room temperature (25-30ºC). The filtered extracts were concentrated under reduced pressure and low temperature in rotary evaporator to get semi solid extracts. Finally, the extracts were analysed by chromatographic and spectroscopic analysis for the identification of various bioactive constituents.

Chromatographic Analysis
Thin layer chromatography
Stem bark extracts of *M. azedarach* (petroleum ether, acetone, ethanol and methanol) was subjected to thin layer chromatographic analysis to determine the presence of bioactive constituents. About 0.1 mg of plant extract was separated on TLC plates using 15% acetone and 85%petroleum ether. The separated components (visible spots) were visualized under visible and ultra violet (UV) light. The qualitative evaluation of the plates was done by determining the migration behavior of the separated substances (visible spots) expressed as a retardation factor (R_f value). TLC is also used for the identification of a compound in a mixture when the R_f of a compound is compared with the R_f of a known compound.

Column chromatography
One ml of methanol extracts was fractionated using Si-gel in a column with the dimension of 28cm×2cm. The eluents are n-hexane and methanol (Eluent ratio; n-hexane/methanol 100%, 80:20, 60:40, 40:60, and 20: 80). The compounds of the bark extract run down to the column forming bands based on their polarity and molecular nature. The eluted volumes were sequentially collected as fractions.

Spectroscopic Analysis
GC-MS spectral analysis
GC-MS analysis was performed on a combined GC-MS instrument (ITQ 900Model of Thermo fisher scientific make) employing the following conditions: HP-5fused silica gel used as capillary column.1µl of sample was injected into the column using PTV injector at 275ºC. The GC program was initiated by a column temperature at 60 ºC for 5 minutes, increased to 300 ºC at a rate of 8 ºC/min, held for 10 minutes. Helium was used as the carrier gas (1.5ml/min). The mass spectrometer was operated in E1 mode with mass source at 200 ºC. The chromatogram and spectrum of the peaks were visualized. The particular compounds presenting the samples were identified by matching their mass spectral fragmentation patterns of the respective peaks in the chromatogram with those stored in the National Institute of Standards and Technology Mass Spectral database (NIST-MS, 1998) library.

UV-VIS and FT-IR analysis
The extract was examined under visible and UV light for proximate analysis. For UV-VIS and FT-IR spectrophotometric analysis, the bark extracts were centrifuged for 15 minutes at 3000 rpm and sieved through Whatman No. 1 filter paper. The diluted (1:10) sample was scanned in the wavelength ranging from 200-1100nm using Perkin Elmer spectrophotometer and the characteristic peak values were detected. FTIR analysis of *M. azedarach* was performed to detect the characteristic peaks and their functional groups.

Results
The chromatographic and spectrometric analysis of stem bark extracts of *M. azedarach* provided the essential preliminary observations, to select crude plant extracts of various solvent with potentially useful properties for further chemical and pharmacological investigations.

Thin layer chromatography
The thin layer chromatographic analysis of *M. azedarach* stem bark extracts showed one visible spot with R_f value of 0.22 in petroleum ether extract: 2 visible spots in acetone and ethanol extracts with R_f values of 0.04, 0.1 and 0.08, 0.26 respectively. A maximum of 3 visible spots were observed in methanolic extract with R_f values of 0.05, 0.10 and 0.25. In accordance with the results obtained, the methanolic extract was considered as best and taken for further analysis. The results were tabulated in Table 1.

Column chromatography

Table 1: TLC profile of *M. azedarach* L.

S. NO	Plant Sample	Solvent extract	No. of peaks	Rf Value
1.	*Melia azedarach* L.	Petroleum ether	1	0.22
		Acetone	2	0.08, 0.26
		Ethanol	2	0.04, 0.11
		Methanol	3	0.05, 0.10, 0.25

The column chromatographical analysis of methanol extract of *M. azedarach* stem bark showed a total of 7 fractions with different solvent run ratio of hexane: methanol (8:2, 6:4, 5:5, 4:6, 2:8) which was collected sequentially and the results were shown in Table 2. A maximum of 7 fractions were obtained in 8:2 solvent run ratio with different colors indicating pale white, snow white, light yellowish green, ochre, brown, light yellow and colourless. Two fractions with snow white and colourless were obtained in 6:4 ratio. One colourless fraction each in 5:5 and 4:6 ratio was obtained. Also, 4 fractions in 2:8 ratio was obtained with, ochre, brown, light yellow and colourless. Whereas, in 100% ethanol and 100% methanol 2 fractions each were obtained.

Table 2: Column Chromatography profile of stem bark extract of *M. azedarach* L.

S.No	Solvent run ratio (Hexane: Methanol)	No. of Fraction	Colour of Fraction
1.	8.2	7	F1- Pale white F2-Snow white F3-Light yellowish green F4-Ochre F5-Brown F6-Light yellow F7- Colourless
2.	6.4	2	F1- Snow white F2- Colourless
3.	5. 5	1	F1- Colourless
4.	4.6	1	F1- Colourless
5.	2.8	4	F1- Ochre F2-Brown F3-Light yellow F4- Colourless
6.	100 % ethanol	2	F1- Light brown F2- Ochre
7.	100 % Methanol	2	F1- Light brown F2- Colourless

GC-MS spectral analysis

The results pertaining to GC- MS analysis led to the identification of various numbers of compounds from GC fractions of the methanolic extracts of *M. azedarach*. They were identified through mass spectrometry attached with GC. GC-MS analysis of methanolic extract of *M. azedarach* was shown in Figure 1. Two major compounds such as 9, 12-tetradecadien-1-o1 in 21.99 RT value and 2-Cyclohexyl-4a, 7-dimethyl-3, 4, 4a, 5, 6, 8a-hexahydro-2H- benzoxaine-3-carboitrile at 33.33 RT value have been detected through GC-MS analysis based on retention time (Table 3).

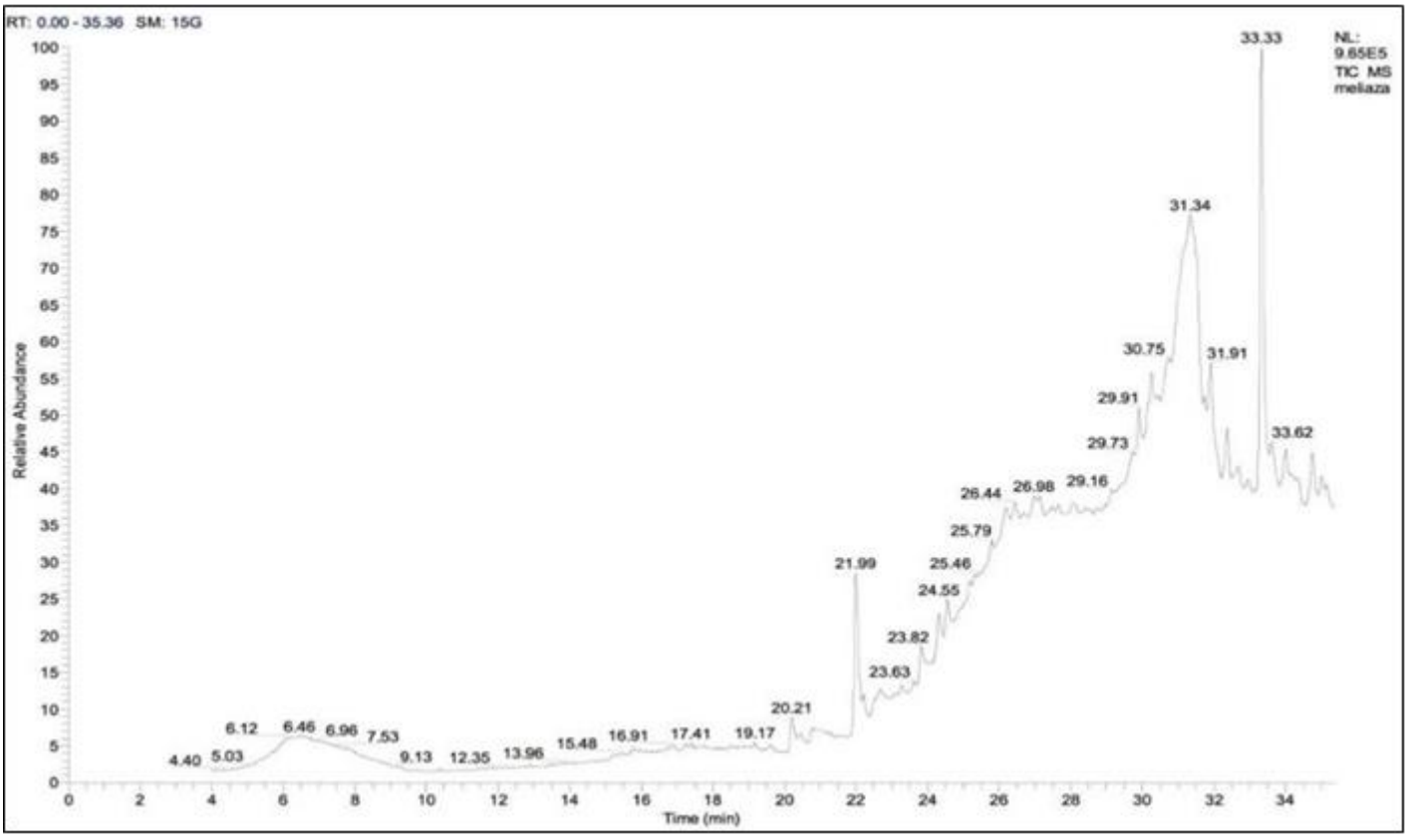

Fig 1: GC-MS Analysis of stem bark methanolic extract of *M. azedarach* L.

Table 3: Phytoconstituents identified as major compounds in stem bark methanol extract of *M. azedarach* by GC-MS.

S. No.	RT	Name of the compound	Molecular Formula	Molecular weight
1.	21.99	Z,E-9,12-Tetradecadien 1-ol	C14H260	210
2.	33.33	2-Cyclohexyl-4a,7-dimethyl-3,4,4a,5,6,8a-hexahydro-24-1,2 benzoxazine-3-carbonitrite	C17H26N20	274

UV-VIS and FT-IR analysis

The methanolic stem bark extract of *M. azedarach* was subjected to UV-Vis and FT-IR analysis in order to find out the functional group identification. The UV-Vis spectrum profile of showed two peaks at 248, and 303 nm with the absorption of -0.020, and 4.856 respectively; the results were showed in Figure 2. The 9 peak values and functional groups of *M. azedarach* obtained from FT-IR spectroscopy were represented in Figure 3. The various peak values of FT-IR indicate the presence of C-Br stretching, C-O stretching, C-N stretching, S=O stretching, C-H stretching, O-H, C-H bending, and N-H stretching, which confirmed the presence of halo compounds, alkyl aryl ether, amine, sulfate, alkanes, alcohols and aliphatic primary amine. The peak values and functional groups obtained was tabulated in Table 4.

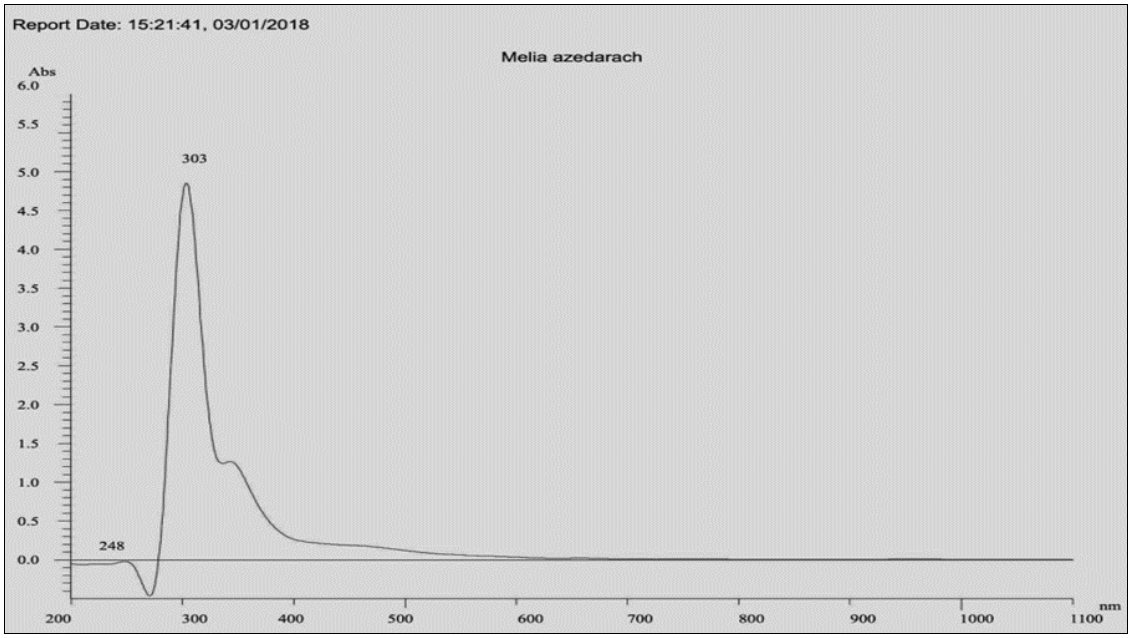

Fig 2: UV Analysis of stem bark methanolic extract of *Melia azedarach* L.

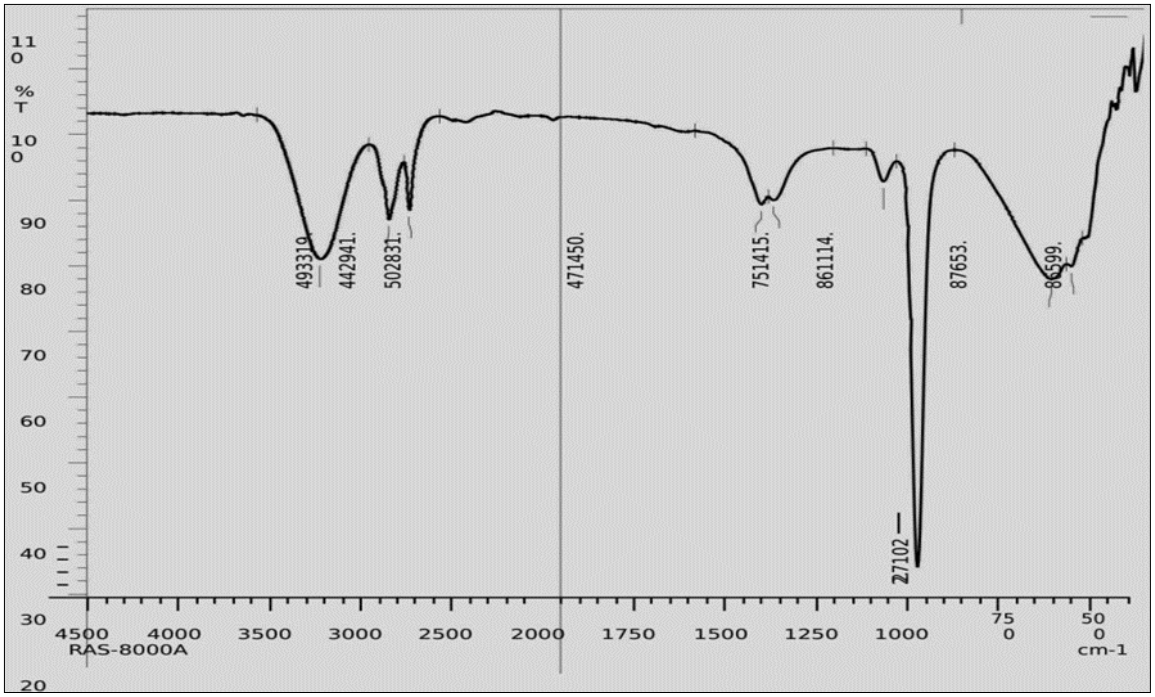

Fig 3: FTIR Analysis of stem bark methanolic extract of *Melia azedarach* L.

Table 4: FTIR peak values and functional groups of stem bark methanol extracts of *M. azedarach* L.

S. No	Peak value	Functional Group	Functional Group Name
1.	3319.49	O-H Stretch	Alcohols
2.	2943.37	C-H Stretch	Alkanes and alkyls
3.	2831.50	O-H Stretch	Carboxylic acid
4.	2526.75	O-H Stretch	Carboxylic acid
5.	1450.75	C-H Bend	Alkanes and alkyls
6.	1415.75	S=O Stretch	Sulfate
7.	1114.86	C-O Stretch	Alcohol
8.	1020.34	C-O-C Symmetrical	Ethers
9.	653.87	C-H Bend	Alkynes
10.	605.65	C-Br Stretch	Alkyl halides
11.	489.92	C-I Stretch	Alkyl halides

Discussion

In the present study, the thin layer chromatographical analysis of *M. azedarach* leaf extracts showed better visible bands in methanol (3 bands), acetone (2 bands), ethanol (2 bands) and petroleum ether (1 band) extracts. The report of *Wrightia tinctoria* leaf extract finds supportive evidence to the present study, where TLC revealed 9 visible spots in ethanol, petroleum ether extracts and 8 visible spots in methanol, acetone extracts [19]. Whereas, in contrary TLC analysis of *Phyla nodiflora* leaf extracts revealed 5 bands in chloroform extract and 1 visible band each in petroleum ether, methanol, ethanol, and acetone extracts, also, 26 compounds were identified in GC-MS analysis with different Rt value [20]. The column chromatographic analysis of the present study showed best result in methanol extract with 7 fractions in hexane: methanol solvent run ratio. Similarly, a group of yellow visible bands with different fractions were obtained in methanolic leaf extract of *M. azedarach* and the fractions were proved to possess cytotoxic effect [21] and 5 fractions in hexane: methanol (8:2) ratio in *W. tinctoria* [19]. Also, from acetone extract of *Curcuma longa*, a phytocompound curcuminoid was isolated by column chromatography in chloroform: methanol (95:5) solvent run ratio with different fractions at different retention time and the individual fractions were determined by UV spectrophotometry [22]. Since retention time of majority of compounds was close to each other, it was very difficult to separate them. Therefore, GC-MS analysis was carried out where 2 major compounds were identified in methanolic extract of *M. azedarach* leaf. But, alternatively 8 compounds were identified in hexane leaf extract of *M. azedarach* [23] and methanolic leaf extract of *W. tinctoria* [19]. Similarly, some fatty acid compounds were reported in methanolic leaf extract of *Cissus quadrangularis* [24]. In contradictory to our report, hexadecanoic acid a component of alcoholic extract was reported in several investigations such as leaves of *Kigelia pinnata* [25]; leaf and stem of *Melissa officinalis* [26]; leaves of *Euphorbia longan* [27].The UV and FT-IR results of the present study finds support with some investigations such as methanolic leaf extract of *Calanthe masuca* [28] and roots of *Selinum vaginatum* [29]. Similar functional groups were reported in methanol root extracts *C. ferruginea* and *C. limon*, with characteristic broad peaks representing alcohol/phenol (OH) functional group. Sharp peaks indicating asymmetric stretching of C-H showed around 2924 cm^{-1} and 2853 cm^{-1} and aromatic/alkene carbon double bonds with wave numbers around 1607 and 1621 cm^{-1} were reported [30]. Also, 4 peaks at 237, 305, 356 and 660 nm with absorption spectra of 0.0055, 4.790, 1.756 and 0.173 in UV profile; also, in FT-IR analysis, ether, alcohol, alkanes, alkyls, carboxylic acids, sulfate, alkynes and alkyl halide groups were reported in methanolic leaf extract of *W. tinctoria* [19].

Conclusion

In accordance with the results obtained, it can be concluded that, stem bark of *M. azedarach* possess a great potential of bioactive phytoconstituents that can cure various diseases. The reports revealed that among the organic solvents used, methanol was more suitable to fractionate the bioactive compounds from the stem bark extracts of *M. azedarach*. The evaluation needs to be carried out for practical and clinical applications for the welfare of mankind. Thus, the present result justifies an importance of reported phytocompounds in biological activity. Also, it provides a fundamental base for further analysis on biological activity and its pharmaceutical importance.

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